THE TOXICITY OF PNEUMONIC LUNGS*

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In lobar pneumonia the toxemia is ascribed largely to toxic substances from virulent pneumococci. Numerous investigations have shown that pneumococci do not produce appreciable amounts of soluble toxin in cultures, although the work of Neufeld and Dold,[1] Rosenow[2] and Cole[3] indicate that a toxic substance is produced from the cocci or contained within them which, when liberated, is capable of producing death in animals with symptoms and lesions suggestive of anaphylaxis. Cole regards this pneumotoxin as preformed in the bacterial cell; the studies of Cohen, Weiss and Kolmer[4] generally confirm these observations, although they experienced considerable difficulty in the preparation of the pneumotoxin. While this toxic substance may be elaborated during lobar pneumonia in sufficient amounts to account in large part for the toxic symptoms in addition to probably influencing enzymic processes, our experiments show that its production in vitro is quite irregular, that large numbers of virulent pneumococci are required for its production, and that relatively large amounts of it are required to produce intoxication of animals. When it is remembered that sections of pneumonic lungs frequently show few pneumococci, it is questionable whether there is a sufficient amount of pneumotoxin produced in lobar pneumonia to account in whole for the toxemia of this disease.

With these considerations in mind we have studied the exudate in lobar pneumonia as a further source of toxic substances. While the investigations of Schenck,[5] Dold,[6] Roger,[7] Wright,[8] and Riesman

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[1] Berl. klin. Wchnschr., 1911, 48, p. 55.
[2] Jour. Infect. Dis., 1911, 9, p. 190.
[3] Jour. Exper. Med., 1912, 16, p. 644.
[4] Jour. Infect. Dis., 1918, 22, p. 476.
[5] Ztschr. f. Immunitätsf., O., 1914, 22, p. 229.
[6] Ibid., p. 561.
[7] Arch. d. Med. Exp. et d'Anat. Path., 1911, 23, p. 37.
[8] Proc. Roy. Irish Acad., 1891-1893, 2, p. 117.

and Kolmer[9] have shown that extracts of various tissues, notably lung tissue, are highly toxic on intravenous injection and produce intravascular coagulation, increase in the coagulation time, fall of blood pressure, etc., yet the pneumonic exudate as a source of toxic substances has not received the attention which we believe it deserves. Riesman[10] has recently drawn particular attention to this subject in a discussion on what he calls the "cellular factor in infectious diseases," and states that in pneumonia the bacterial toxemia has been overemphasized to the exclusion of everything else, whereas it is not improbable that some of the general symptoms are due to the exudate, independently of the bacteria. The work of Rosenow and Arkin[11] indicates that the exudate in lobar pneumonia is toxic in that extracts of pneumonic lungs in salt solution injected intravenously in dogs produced blood pressure and respiratory disturbances strikingly similar to those of protein anaphylaxis in general.

THE TOXICITY OF EXTRACTS OF NORMAL AND PNEUMONIC LUNGS

Lungs secured soon after death, and in the stage of gray hepatization, were cut in small pieces, washed and the consolidated parts passed through a meat grinder; this material was then subjected to high pressure in a Buchner hydraulic press, the juice centrifuged at high speed, and the fluid filtered through sterile paper. Almost invariably these extracts contained pneumococci and other bacteria capable of killing animals in small doses; for this reason they were sterilized with phenol up to 0.5% or the fluids were recentrifuged for an hour at high speed and heated at 56 C. for 30 minutes. Marked differences in toxicity followed these two methods of preparation, the first method yielding products which were invariably more toxic than the second. The more ideal method of securing the toxic substances of the lung tissue and exudate by passing the juice through a sterile Berkefeld filter to remove the pneumococci, was unsuitable inasmuch as the toxic substances were largely removed by the filtration.

The fatal toxic dose of pneumonic lung extract prepared by phenol sterilization was about 4 c c per kilo of weight when injected intraperitoneally in mice; the toxicity of fresh extract sterilized by thorough centrifuging and heating at 56 C. for 30 minutes, was between 15 and 25 c c per kilo of weight (Table 1).

By intravenous injection in guinea-pigs, doses of phenolized extract greater than 0.5 c c per kilo of weight were almost invariably fatal within a few minutes producing convulsions and dyspnea similar to acute anaphylactic intoxication (Table 2). Table 2 also shows that intravenous injections of the phenolized extracts in rabbits usually resulted in death within a few minutes in doses greater than 0.12 c c per kilo of weight. Repeated intramuscular injections of these extracts in rabbits, produced fever and rapid loss of weight; doses greater than 0.8 c c per kilo of weight usually produced death after 2 or 3 injections.

[9] Trans. Assoc. of Amer. Phys., 1912.
[10] Trans. College of Phys. of Phila., 1914, 36, p. 271.
[11] Jour. Infect. Dis., 1912, 11, p. 480.

TABLE 1

TOXICITY OF EXTRACT OF HUMAN PNEUMONIC LUNG FOR MICE BY INTRAPERITONEAL INJECTION

Weight in Gm.	Dose per Kilo in c c	Kind of Extract	Results
17	25	Heated	Died in 48 hours—heart blood sterile
24	15	Heated	Survived
25	7.5	Heated	Survived
21	5	Heated	Survived
19	4	Heated	Died in 48 hours—heart blood sterile
19	2	Heated	Survived
13	1	Heated	Survived
17	0.5	Heated	Died in 24 hours—heart blood sterile
13.5	16	Phenolized	Died in 24 hours
20	8	Phenolized	Died in 24 hours
15	4	Phenolized	Died in 24 hours
20.5	2	Phenolized	Survived
20	1	Phenolized	Survived
20.5	0.5	Phenolized	Survived

TABLE 2

TOXICITY OF EXTRACT OF HUMAN PNEUMONIC LUNG FOR GUINEA-PIGS AND RABBITS BY INTRAVENOUS INJECTIONS

Weight in Gm.	Dose in c c per Kilo	Results
Guinea-pig 215	2.3	Died in one minute in convulsions
Guinea-pig 190	1.0	Died in convulsions in 10 minutes
Guinea-pig 225	0.7	Died in 24 hours—no immediate symptoms
Guinea-pig 195	0.5	Survived—no symptoms
Rabbit 2350	0.5	Died in one minute—convulsions
Rabbit 2200	0.25	Died in two minutes—convulsions
Rabbit 2800	0.12	Restless, survived; lost weight

That the toxicity of the extracts cannot be ascribed entirely to the cellular and serofibrinous exudate, is shown by the toxicity of extracts of normal human lungs prepared in the same manner and of equal weights of the respective tissues. The anterior lobes of normal human lungs secured shortly after death, extracted and phenolized in the same manner as the pneumonic tissues, proved about one-half to one-fifth as toxic for various animals after injection by various routes as similar extracts of pneumonic tissue (Table 3).

TABLE 3

COMPARATIVE TOXICITY OF EXTRACTS OF NORMAL AND PNEUMONIC HUMAN LUNGS

Extract of Normal Lung			Extract of Pneumonic Lung		
Weight in Gm.	Dose in C C per Kilo	Result	Weight in Gm.	Dose in C C per Kilo	Result
21	20	Died in 48 hours	20	10	Died in 24 hours
22	15	Died in 24 hours	24	8	Died in 24 hours
16	10	Survived	23	4	Survived
10	8	Survived	17	1	Survived

As the extracts of pneumonic lungs were more toxic than similar preparations of normal lungs, we undertook to analyze this phenomenon and to determine, if possible, to what element, pneumotoxin—that is, the poison derived from autolyzed pneumococci—or poison of the inflammatory exudate. this toxicity is due.

Dogs were infected with concentrated broth cultures of virulent pneumococci by intrabronchial insufflation (Lamar and Meltzer).[12] By means of a proper catheter, 20 c.c. of a 12-24-hour broth culture of virulent Type I pneumococci were injected intrabronchially; 0.000,001 of 24-hour culture killed mice. In each experiment a second dog was given an equal amount of a thick sterile emulsion of aleuronat or a sterile saturated aqueous solution of commercial peptone, in exactly the same way. Forty-eight hours later these dogs as well as a normal control, were killed, the lungs extracted and prepared by the two methods described. With virulent pneumococci, extensive areas of consolidation, as described by Lamar and Meltzer, were invariably observed; aleuronat and peptone produced more irregular consolidation and the lesions resembled more closely those described by Riesman and Kolmer as due to pneumococci of lesser virulence, and by Wollstein and Meltzer as produced by heat-killed pneumococci and sterile broth. The results, while irregular, indicated clearly, as shown by Table 4, that the sterile extracts of consolidated lung due to virulent pneumococci were more toxic than extracts of aleuronat consolidation and of normal lung; likewise extracts of aleuronat exudate were more toxic than the extracts of normal lung (Table 5).

TABLE 4

COMPARATIVE TOXICITY OF PHENOLIZED EXTRACTS OF NORMAL AND CONSOLIDATED DOG LUNGS FOR MICE ON INTRAPERITONEAL INJECTION

Extract of Pneumococcus Consolidated Lung			Extract of Aleuronat Consolidated Lung			Extract of Normal Lung		
Weight in Gm.	Dose per Kilo in C C	Result	Weight in Gm.	Dose per Kilo in C C	Result	Weight in Gm.	Dose per Kilo in C C	Result
20	32	Died in 24 hours	19	25	Survived	20	50	Died in 72 hours
27	16	Died in 24 hours	..	..	....	15	30	Survived
20	8	Survived	..	..	....	..	..	....

TABLE 5

COMPARATIVE TOXICITY OF PHENOLIZED EXTRACTS OF ALEURONAT EXUDATE IN DOG LUNG AND NORMAL DOG LUNG BY INTRAVENOUS INJECTION IN GUINEA-PIGS

Extract of Aleuronat Lung			Extract of Normal Lung		
Weight in Gm.	Dose per Kilo in C C	Result	Weight in Gm.	Dose per Kilo in C C	Result
215	2	Died in 24 hours	250	5	Died in 48 hours
255	1	Survived	260	4	Survived
...	..	.....	250	1	Survived

The toxicity of extracts of pneumonic lung in gray hepatization and of normal lung is slightly decreased by heating the extracts at 60 C. for 1 hour; the pneumotoxin, that is, the toxic substance obtained by autolysis of virulent pneumococci, is, however, extremely thermolabile. The toxicity of one extract

[12] Jour. Exper. Med., 1912, 15, p. 133.

reheated at 60 C. for 1 hour was appreciably decreased; similar experiments with other extracts prepared by phenol sterilization showed that exposure at 56 C. for 1 hour had less effect, so that the toxic factors may be regarded as largely thermostabile.

Thorough drying of the extracts of normal and pneumonic lungs (in gray hepatization) at 19 C. by means of an electric fan, followed by emulsification of the powder in sterile salt solution and injection intraperitoneally in mice, gave a similar decrease in toxicity in experiments so conducted that the doses of dried substances corresponded to the extracts in the fluid state before drying.

Filtration of the extracts through sterile filter paper of medium density does not alter appreciably their toxicity; filtration through small unglazed porcelain (Kitasato) and Berkefeld N filters appreciably reduces the toxicity, but does not remove it entirely, as occurs on the filtration of the pneumotoxin alone through similar filters.

As shown by Cole[3] the pneumotoxin obtained by autolysis of virulent pneumococci in solutions of sodium choleate, is frequently hemolytic for the erythrocytes of the guinea-pig and other animals; in the experiments of Cohen, Weiss and Kolmer[4] it was found difficult to prepare this hemolytic substance but a sufficient number of experiments were successful to confirm Cole's observations and indicate the hemolytic nature of the endocellular toxin of the pneumococcus. Similar results were observed with extracts of pneumonic lung in gray hepatization, that is, some extracts were markedly hemolytic for guinea-pig cells and others were not hemolytic in large amounts; like the pneumotoxin, the hemolytic effect in extracts of pneumonic lung was reduced by heating and drying and usually completely removed by filtration through Berkefeld filters (Table 6). Similar extracts of normal human lung tissue were found to have little or no hemolytic effect and extracts of the exudates of lungs of dogs removed 48 hours after the intrabronchial injection of virulent pneumococci and of sterile aleuronat, were generally nonhemolytic, indicating that in fresh pneumococcus exudates appreciable amounts of hemolytic substance are not present but present in human pneumonic lungs in gray hepatization and with early autolysis of the exudate.

TABLE 6

THE HEMOLYTIC ACTIVITY OF PHENOLIZED EXTRACTS

Extract	Dilution of Extract	Results
Extract of human pneumonic exudate	1:100	Marked lysis with 0.2 c c, complete lysis with 0.3 c c of substance
Extract of normal human lung.....	1:50	Very slight lysis with as much as 2.0 c c of substance
Extract of pneumonic exudate heated at 56 C. for 60 minutes	1:100	No lysis with 0.7, marked with 0.8, and complete with 0.9, c c of substance
Extract of pneumonic exudate, dried, emulsified in salt solution	1:100	No lysis below 0.5, complete lysis with 0.7 c c of substance
Dried extract of normal lung.....	1:50	No lysis
Berkefeld filtrate of pneumonic exudate	1:100	No lysis
Extract of pneumococcus exudate in lung of dog (48 hours)	1:10	No lysis
Extract of aleuronat exudate in lung of dog (48 hours)	1:10	No lysis
Extract of normal dog lung.....	1:10	No lysis

The nature of the hemolytic substance sometimes present in extracts of pneumonic lungs in the later stages of pneumonia, is unknown; our experiments indicate that this agent may be neutralized not only by antipneumococcus serum corresponding to the type of the pneumococcus infection, but also by normal serum, e. g., rabbit. It is probable that the lipoidal constituents of these serums act as antihemolytic agents and additional experiments indicate that the presence of lipoids in the lung extracts decrease their hemolytic activity inasmuch as the removal of lipoids from dried pneumonic tissue exudates, is followed by an increase of hemolytic activity.

Cole[13] has shown recently that the fluid of pneumococcus empyema may contain large amounts of soluble substances which have the property of neutralizing pneumococcus antibodies; since then we have had the opportunity of studying the influence of one extract from pneumonic lung in gray hepatization, obtained by grinding the tissues and by high pressure in a hydraulic press followed by thorough centrifugation and filtration through sterile paper. This extract was not heated and was used at once without preservative. A similar extract was prepared of an equal weight of normal human lung tissue. Both extracts were tested for inhibition of agglutination of Type I pneumococci by homologous antiserum (Rockefeller Institute) by the technic described by Cole. While 0.4 c c of serum diluted 1:60 produced well marked agglutination of 0.1 c c of a broth culture of pneumococci, the addition of 0.4 c c of pneumonic lung extract reduced the agglutinating influence of the serum to a dilution of 1:20 and the extract of normal lung to a dilution of 1:40 (Table 7).

TABLE 7

THE INHIBITION OF AGGLUTINATION OF PNEUMOCOCCI BY ANTIPNEUMOCOCCUS SERUM BY THE EXTRACTS OF PNEUMONIC AND NORMAL LUNG *

Extract	Dilution of Immune Serum							
	1:2	1:4	1:8	1:20	1:40	1:60	1:80	1:120
Pneumonic lung.....	+	+	+	+	—	—	—	—
Normal lung.....	+	+	+	+	+	—	—	—
Control—salt solution.....	+	+	+	+	+	+	+	+

* Mixtures of 0.4 c c of lung extracts or salt solution with 0.4 c c of immune serum, undiluted and diluted, were made in small tubes and placed at 37 C. for 30 minutes when 0.1 c c of a 24-hour broth culture of Type I pneumococci was added to each tube; the tubes were then placed in incubation for one hour and in cold box over night.

While this result requires further work before definite conclusions can be drawn, it would appear that extracts of lung tissue and particularly of pneumonic lung in gray hepatization, contains substances capable in slight degree of neutralizing the agglutinin in antipneumococcus serum similar to those found by Cole in pneumococcus empyema fluids.

CONCLUSIONS

Salt solution extracts of pneumonic lung in the stage of gray hepatization were found to be more toxic for animals than similar extracts of normal lung tissue. The method of extraction influenced the toxicity of both extracts.

[13] Ibid., 1917, 26, p. 453.

Lethal doses of extracts of both pneumonic and normal lung injected intravenously usually produced anaphylactic symptoms.

Sterile extracts of pneumonic lung in dogs removed 48 hours after intrabronchial insufflation of virulent pneumococci, were more toxic than similar extracts of lung consolidated by intrabronchial insufflation of sterile aleuronat, and both were more toxic than extracts of equal weights of normal dog lung.

The toxicity of extracts of normal and pneumonic lung is decreased by heating, drying and filtration through Berkefeld filters.

Extracts of human pneumonic lungs in gray hepatization were frequently hemolytic for guinea-pig cells, whereas similar extracts of normal human and dog lungs and of dog lungs consolidated from intrabronchial insufflation of virulent pneumococci and sterile aleuronat, were generaly nonhemolytic. The hemolytic activity of these extracts was neutralized by antipneumococcus serum as well as by normal rabbit serum; reduced by heating and drying, and usually completely removed by porcelain filtration.

One extract of a human pneumonic lung in gray hepatization was found to partly neutralize the agglutinating activity of an antipneumococcus serum.

The nature of the toxic and hemolytic substance or substances in extracts of pneumonic lung is unknown; it is probably allied with the protein fractions and may be partly responsible for the production of the various systemic symptoms of lobar pneumonia ascribed to toxemia.